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Towards future-proof crop protection in Europe

Towards diagnosis-treatment combinations

Hans Helder, Wageningen University (NL)

EFPP
European Foundation for Plant Pathology

- Diagnosis-treatment combinations (DTC)
  - A relatively new concept in healthcare
    - in The Netherlands
- (On January 1, 2005, it was introduced as a new financing system for hospitals).

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- A DTC includes all the activities and actions performed by the hospital and medical specialist in response to the patient’s need for care, from the first consultation or examination to the final checkup.

- A DTC gives better insight into treatments performed by hospitals and the cost of such treatments, so that healthcare insurers know exactly what they are paying for and can compare hospitals and specializations.

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Diagnosis – treatment combinations in plant pathology

Diagnosis: Pathogens		Treatment: Handles in IPM
virus		Culture measures (*e.g.* (non-) tillage, crop rotation, etc.)
bacteria		PAMP-triggered immunity (PTI) Basal resistance
phytoplasmas		Effector-triggered immunity (ETI) Host plant resistance
oomycetes		Management general and specific (soil) suppressiveness
fungi		Biological control
nematodes		Pheromones
insects		*Agrochemicals*

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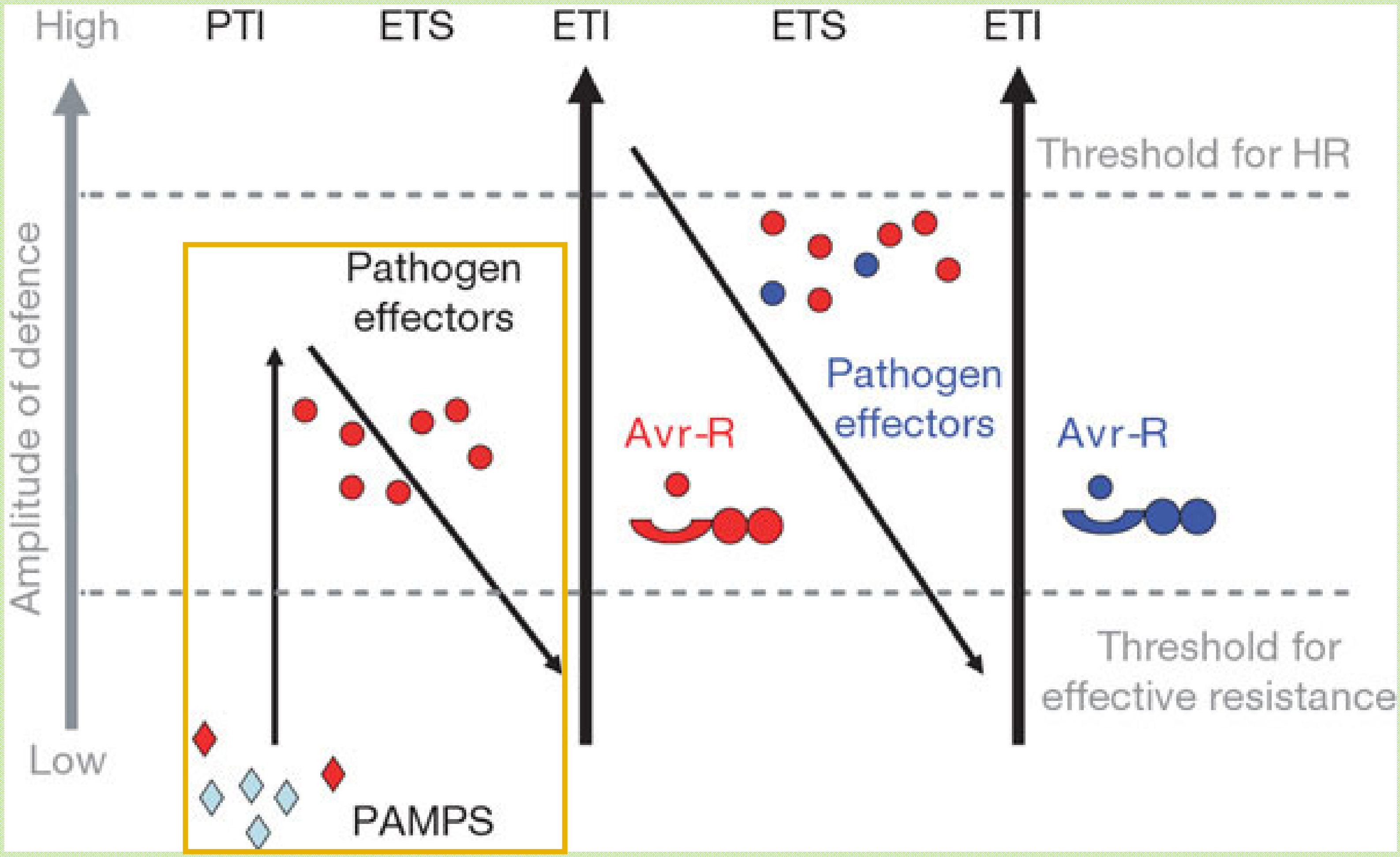
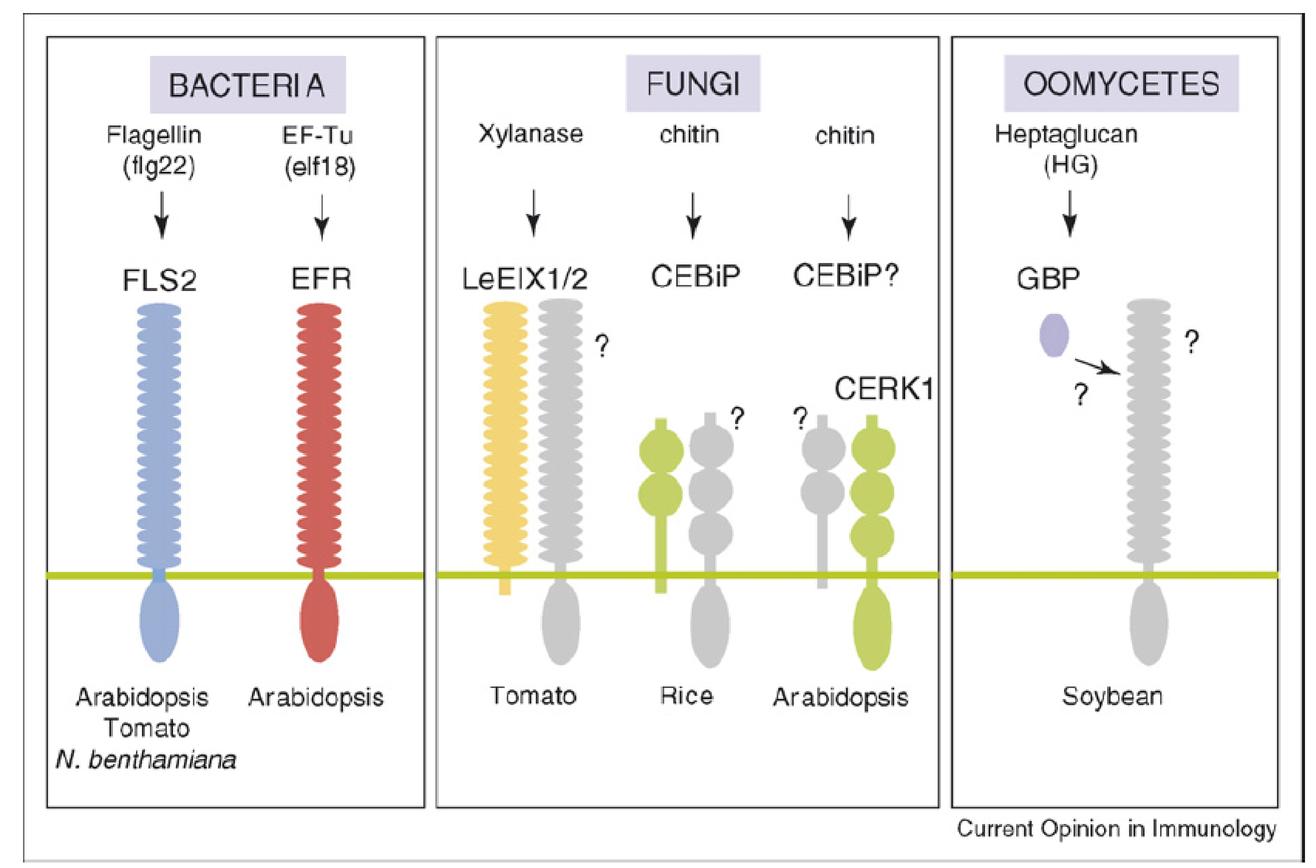


Jones and Dangl (2006)

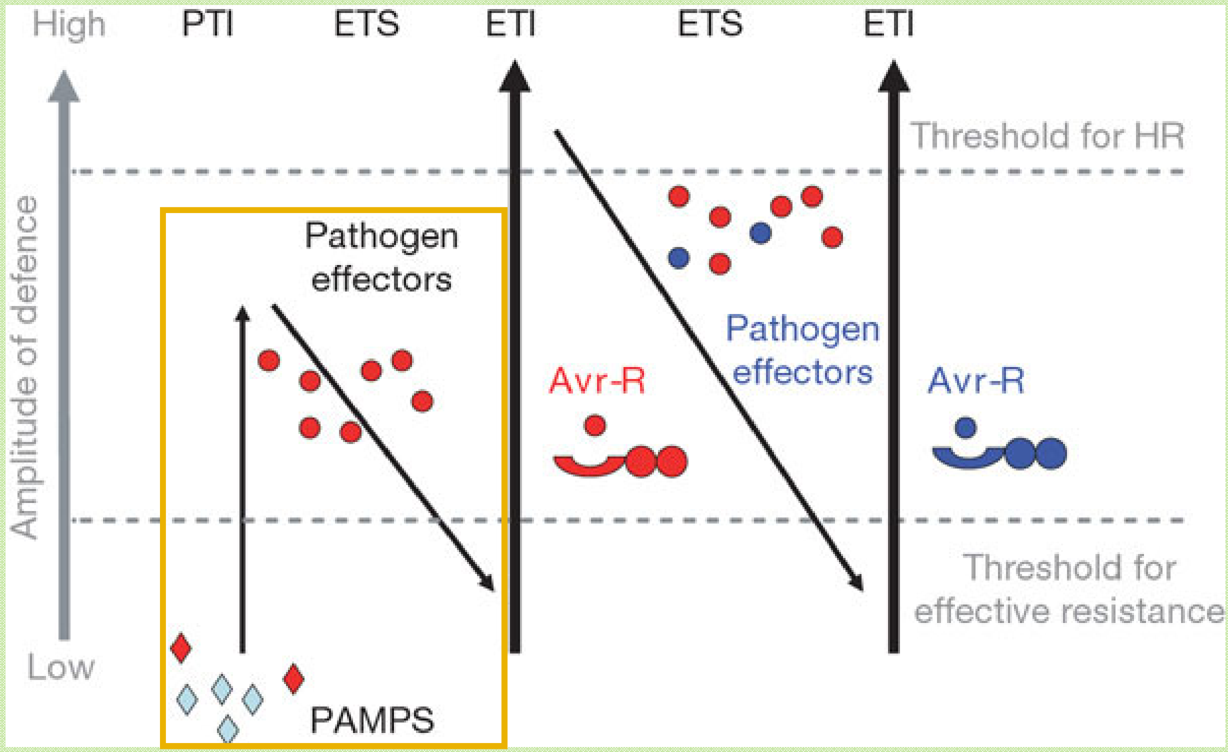
1. Plants detect microbial/pathogen-associated molecular patterns (MAMPs/PAMPs, red diamonds) via PRRs to trigger PAMP-triggered immunity (PTI) that can halt further colonization.

PRRs: transmembrane pattern recognition receptors



Cyril Zipfel (2008)

Plant PRRs. Bacterial flagellin (flg22) and EF-Tu (elf18) are recognized by the LRR-RLKs FLS2 and EFR, respectively. Orthologues of FLS2 have been cloned and characterized in tomato and *N. benthamiana*. In tomato, xylanase is recognised by the RLPs LeEIX1 and LeEIX2. LRR: leucine-rich repeat (leucine: hydrophobic amino acid); RLK: receptor-like protein kinase



Jones and Dangl (2006)

PAMP-triggered immunity (PTI) -formerly named
basal or non-cultivar-specific resistance
(to be compared with animal innate immunity).

= Ancient & conserved first layer of defense, it is based on the perception of conserved microbial structures by PRRs and it is effective against a broad spectrum of invading microorganisms

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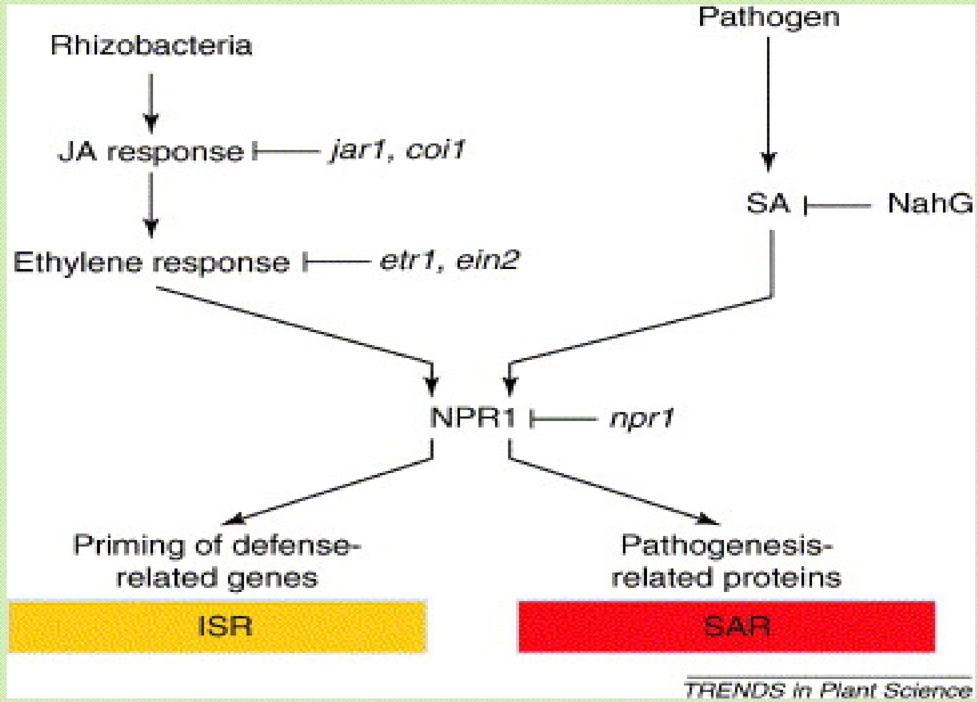
How to measure PAMP-triggered immunity (PTI)? NPR1 as an example



Signal transduction pathways of SAR and ISR.

SAR – Systemic acquired resistance (induced by an avirulent pathogen or upon restricted infection by a virulent pathogen

ISR – Induced systemic resistance (induced by non-pathogenic rhizobacteria)

NPR1 – non-expressor of PR genes

A functional NPR1 protein is required for ISR and SAR – marker for basal resistance

From: L.C. van Loon , Bart P.J., Geraats, Huub J.M., Linthorst (2006)
Trends in Plant Science 11 (4) 184 - 191

OK, but are these *Arabidopsis* findings relevant for crops?

RESEARCH PAPER

Malus hupehensis NPR1 induces pathogenesis-related protein gene expression in transgenic tobacco

J.-Y. Zhang[1,2], Y.-S. Qiao[1], D. Lv[1], Z.-H. Gao[1], S.-C. Qu[1] & Z. Zhang[1]
1 College of Horticulture, Nanjing Agricultural University, Nanjing, China
2 Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences, Nanjing, China

Research article

Open Access

Characterization of _Vitis vinifera_ NPR1 homologs involved in the regulation of _Pathogenesis-Related_ gene expression

Gaëlle Le Henanff[1], Thierry Heitz[2], Pere Mestre[3], Jerôme Mutterer[2], Bernard Walter[1] and Julie Chong*[1]

Functional analysis of the *Theobroma cacao NPR1* gene in *arabidopsis*

Zi Shi[1], Siela N Maximova[2], Yi Liu[1], Joseph Verica[2], Mark J Guiltinan[1,2*]

Etc., etc.

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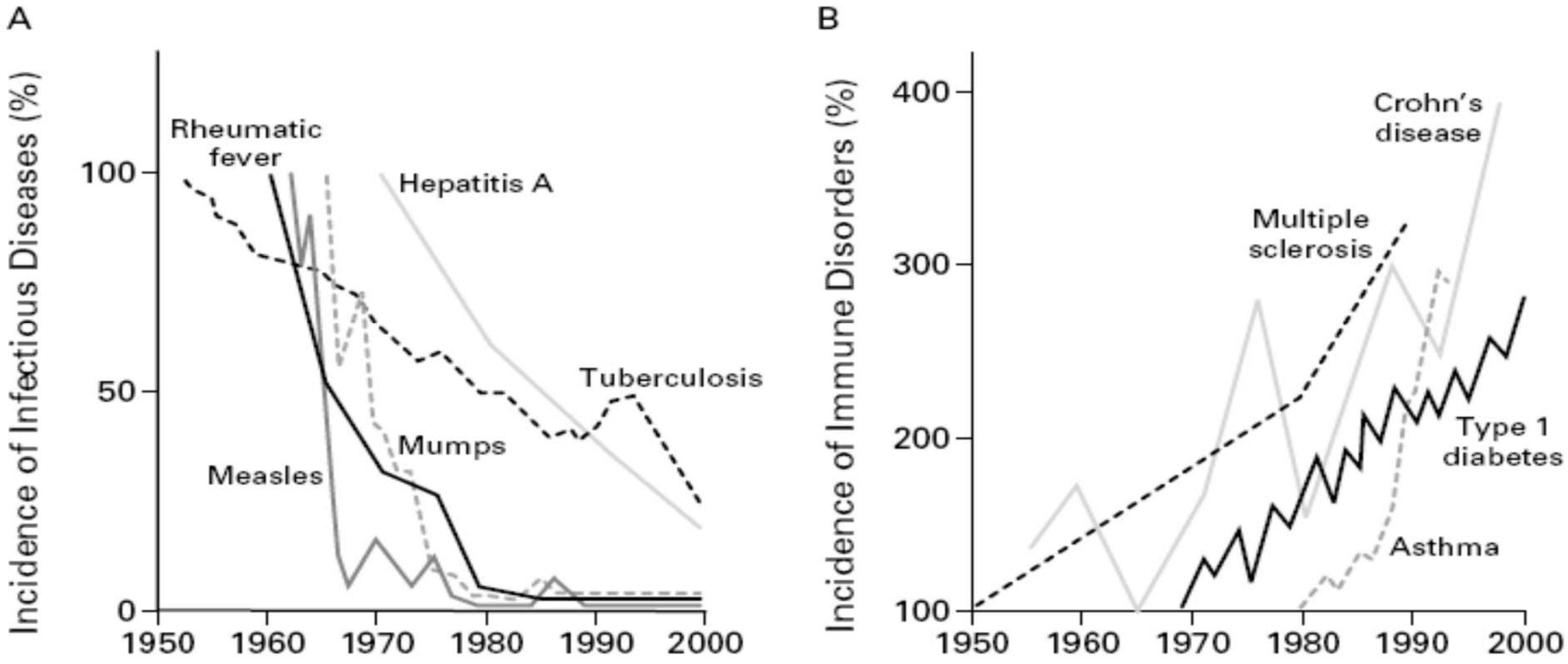
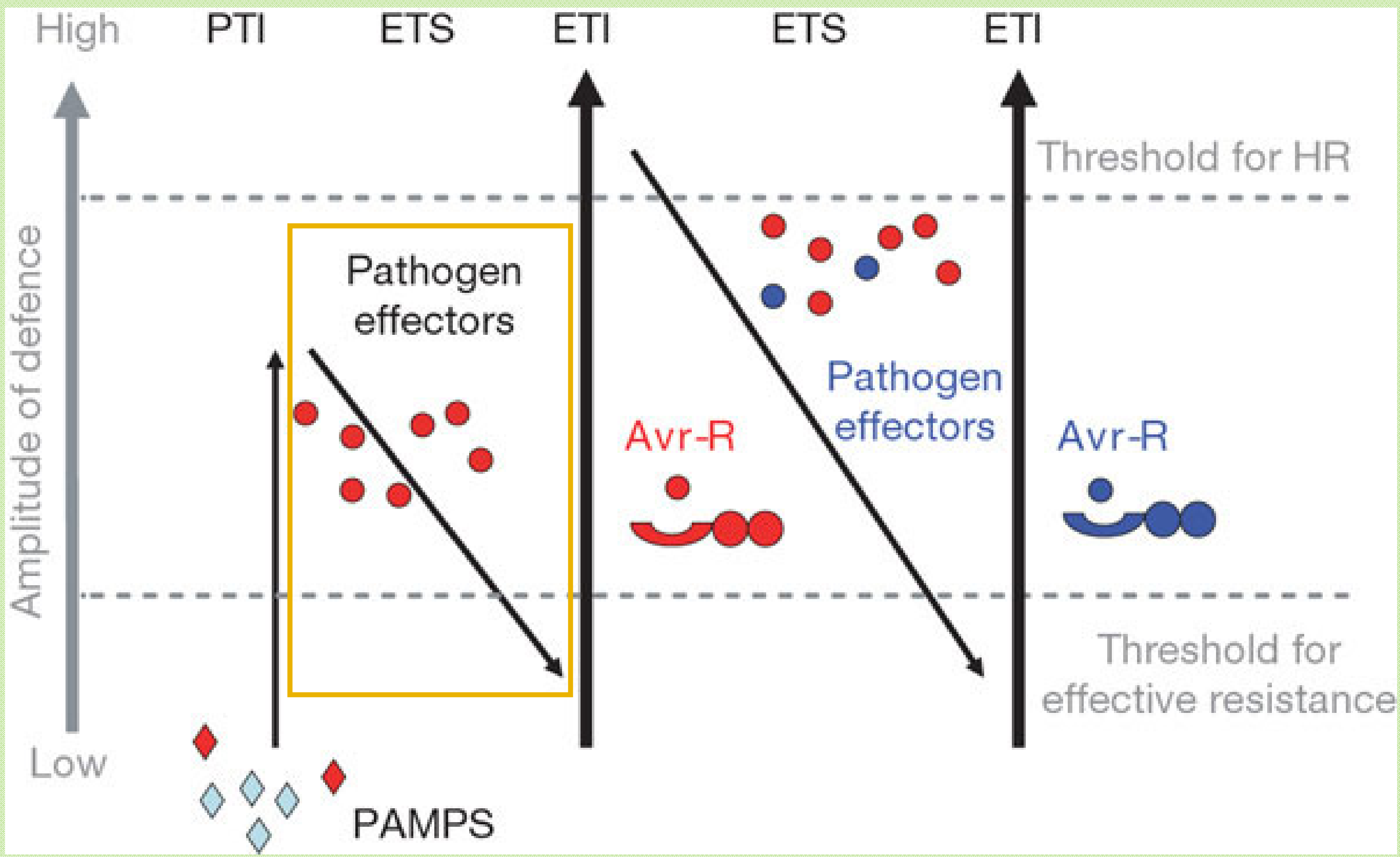
Inverse relation infectious diseases and immune disorders: hygiene hypothesis

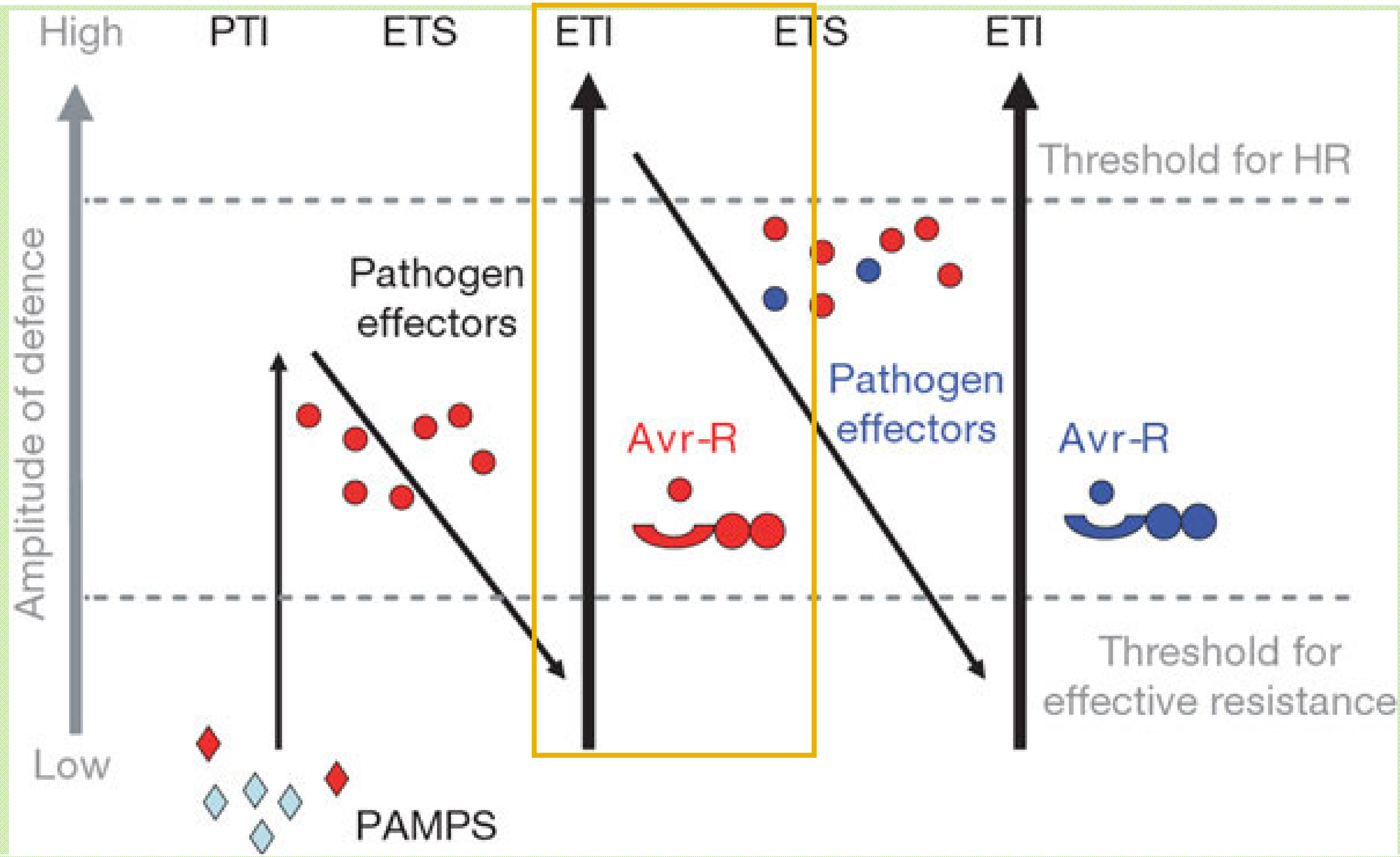


N Engl J Med 2002

The developing immune system must receive stimuli (from infectious agents, symbiotic bacteria, or parasites) in order to adequately develop regulatory T cells

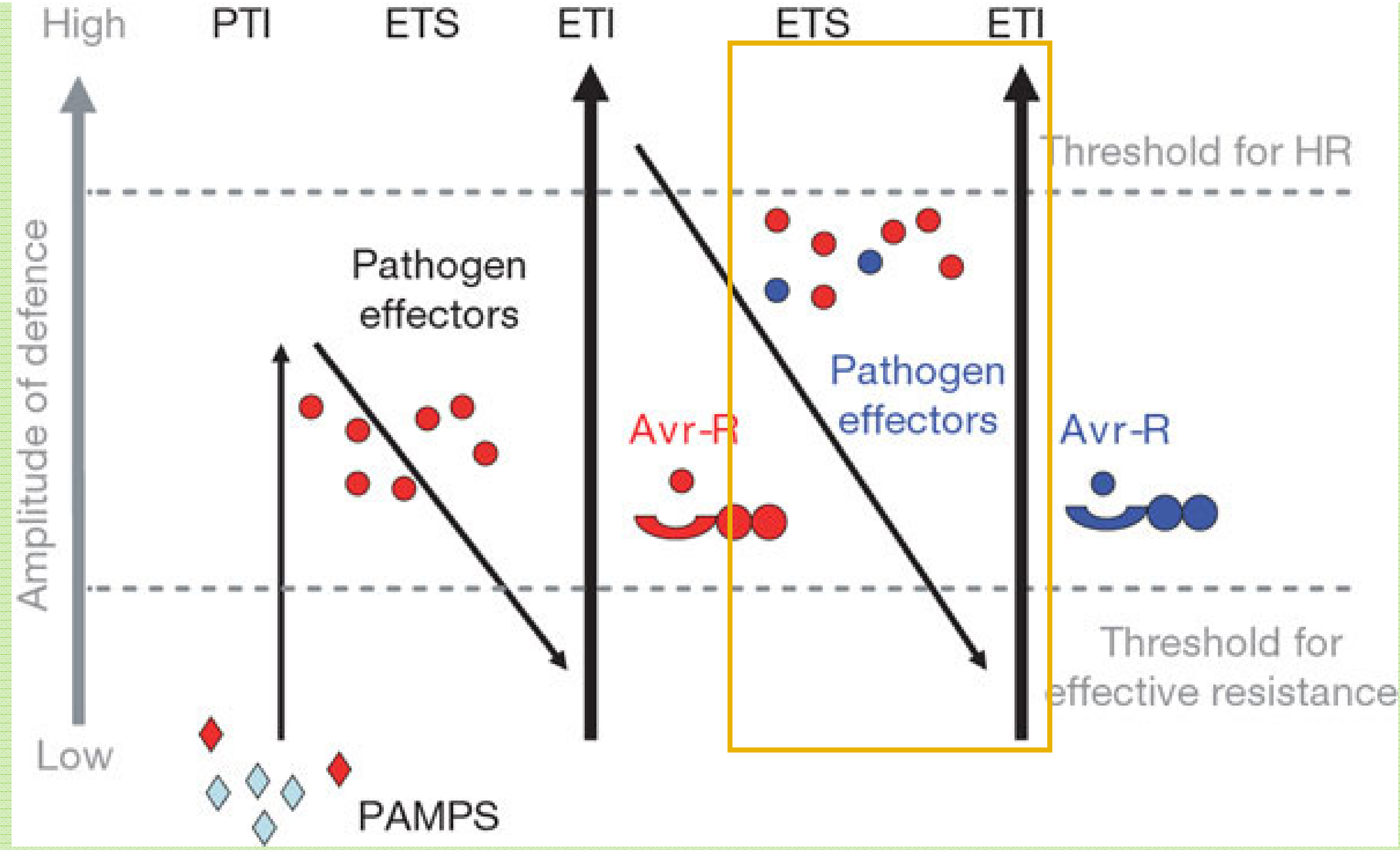


2. Successful pathogens deliver effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS).



3. A given effector is 'specifically recognized' by one of the NB-LRR proteins, resulting in effector-triggered immunity (ETI).

ETI is an accelerated and amplified PTI response



Jones and Dangl (2006)

4. Natural selection drives pathogens to avoid ETI either by shedding or diversifying the recognized effector gene, or by acquiring additional effectors that suppress ETI. Natural selection results in new *R* specificities so that ETI can be triggered again.

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Effector-triggered immunity (ETI)

Resistance proteins

→ highly specific

→ strong response

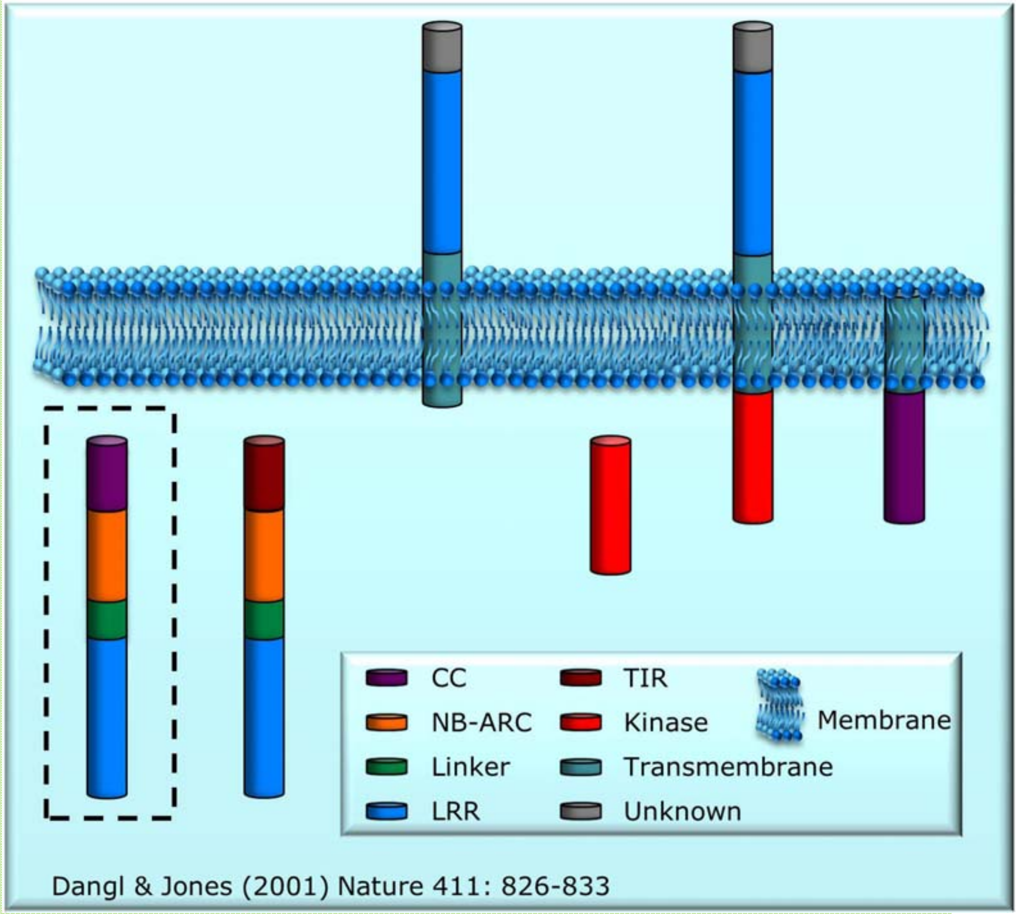
→ divided in classes based on their domains

NB-ARC-LRR

→ the most common R proteins

→ ~ 800 in potato genome

→ N-terminal CC or TIR



ETI, Example 1: virus and nematode

Two highly similar R proteins recognize two very different pathogens

Rx1

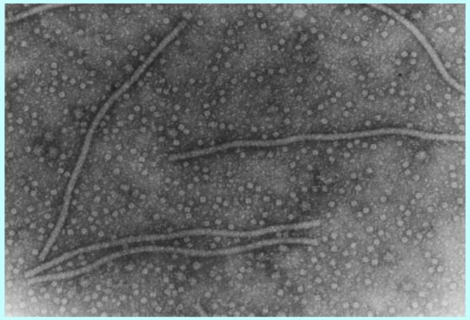
Extreme resistance to Potato Virus X

Elicitor: PVX Coat protein

Gpa2

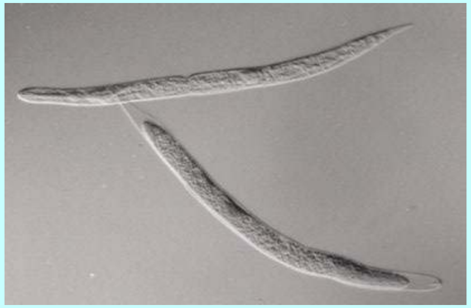
Slow response to *G. pallida* D383

Elicitor: secreted RanBP-like protein



Virus particle: l = 515 nm
□ = 13 nm

PVX symptoms on *N. benthamiana*



Length ~500 µm

Cysts on roots
www.eppo.org

ETI, Example 1: virus and nematode

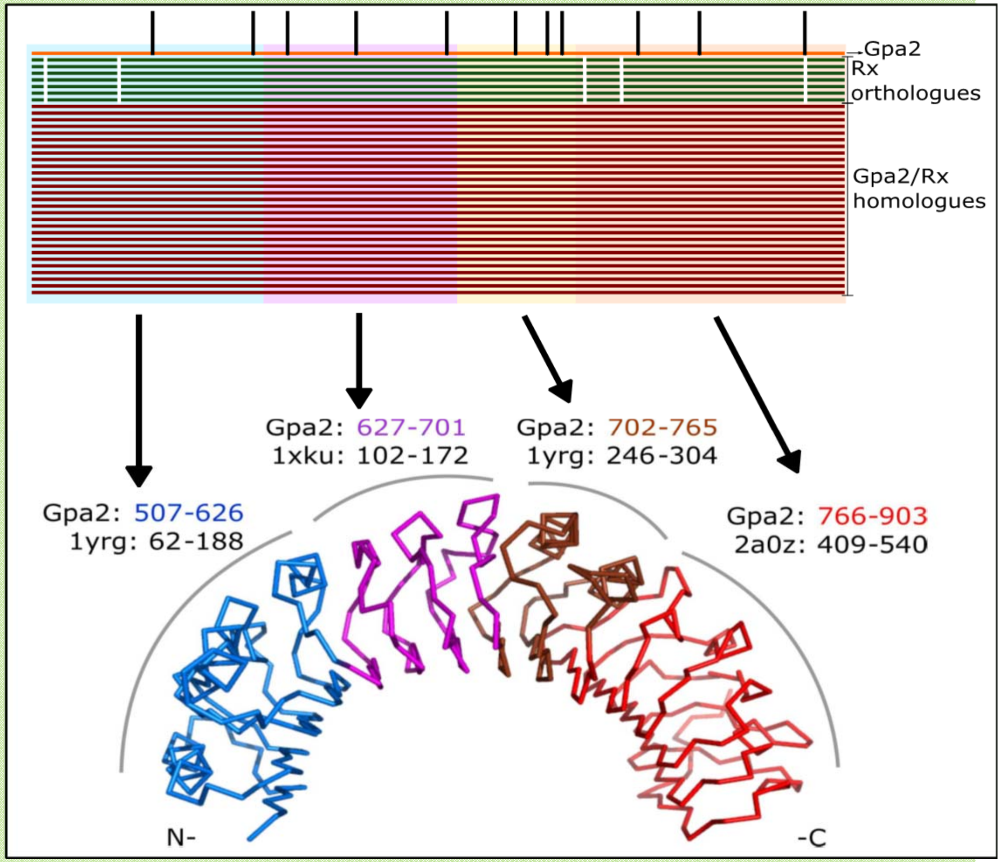
Full length dataset used to calculate 3D model of Gpa2/Rx LRR

Gpa2 LRR is modeled after multiple templates

→ Too irregular for single template

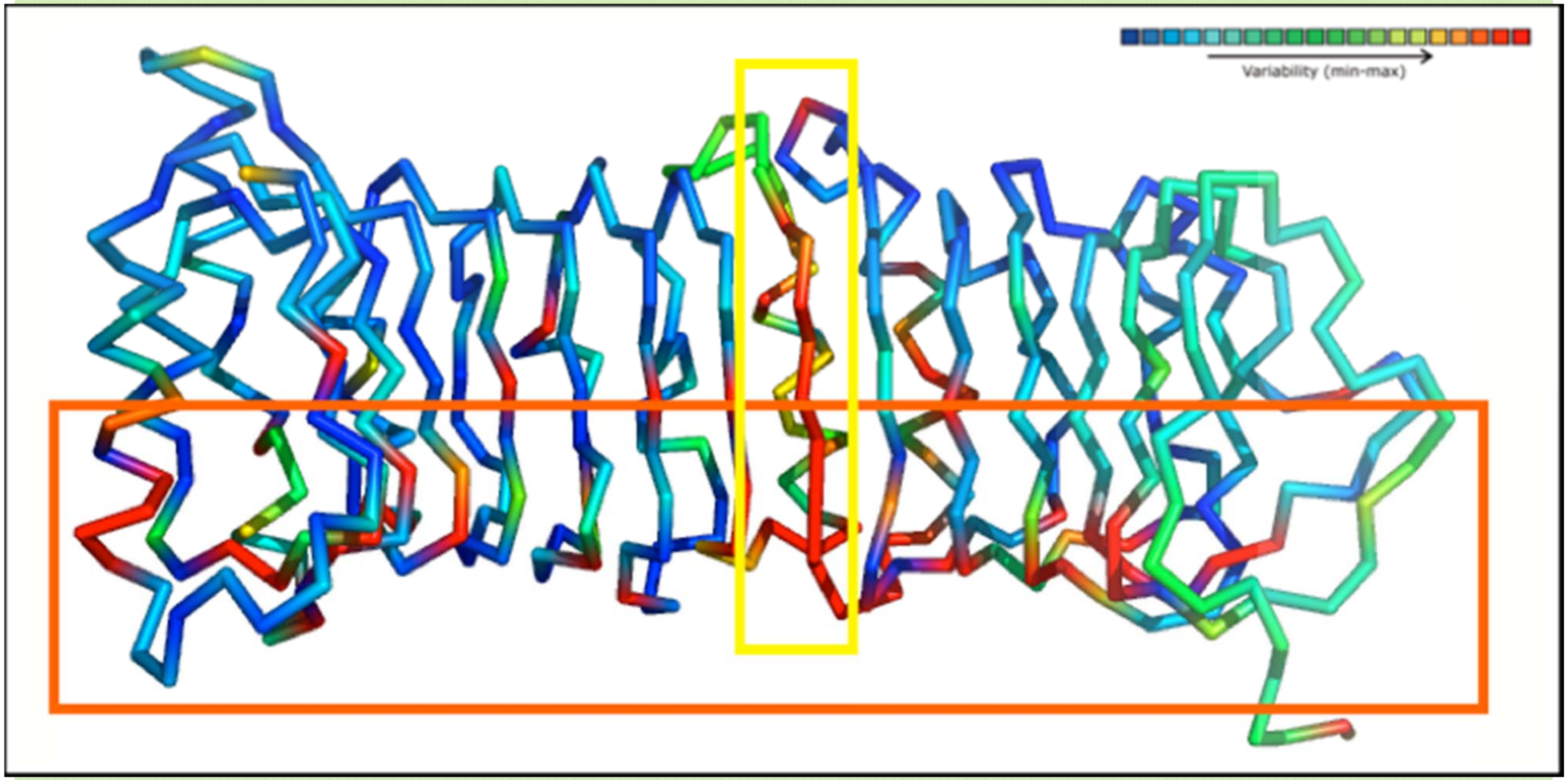
→ Best structural template chosen per LRR segment

- RanGAP rna1p (1yrg),
- TLR3 (2a0z),
- Decorin (1xku)



ETI, Example 1: virus and nematode

Diversity of Gpa2/Rx homologs



Available online at www.sciencedirect.com

SciVerse ScienceDirect

ELSEVIER

Current Opinion in Plant Biology

How to build a pathogen detector: structural basis of NB-LRR function

Frank LW Takken[1,3] and Aska Goverse[2,3]

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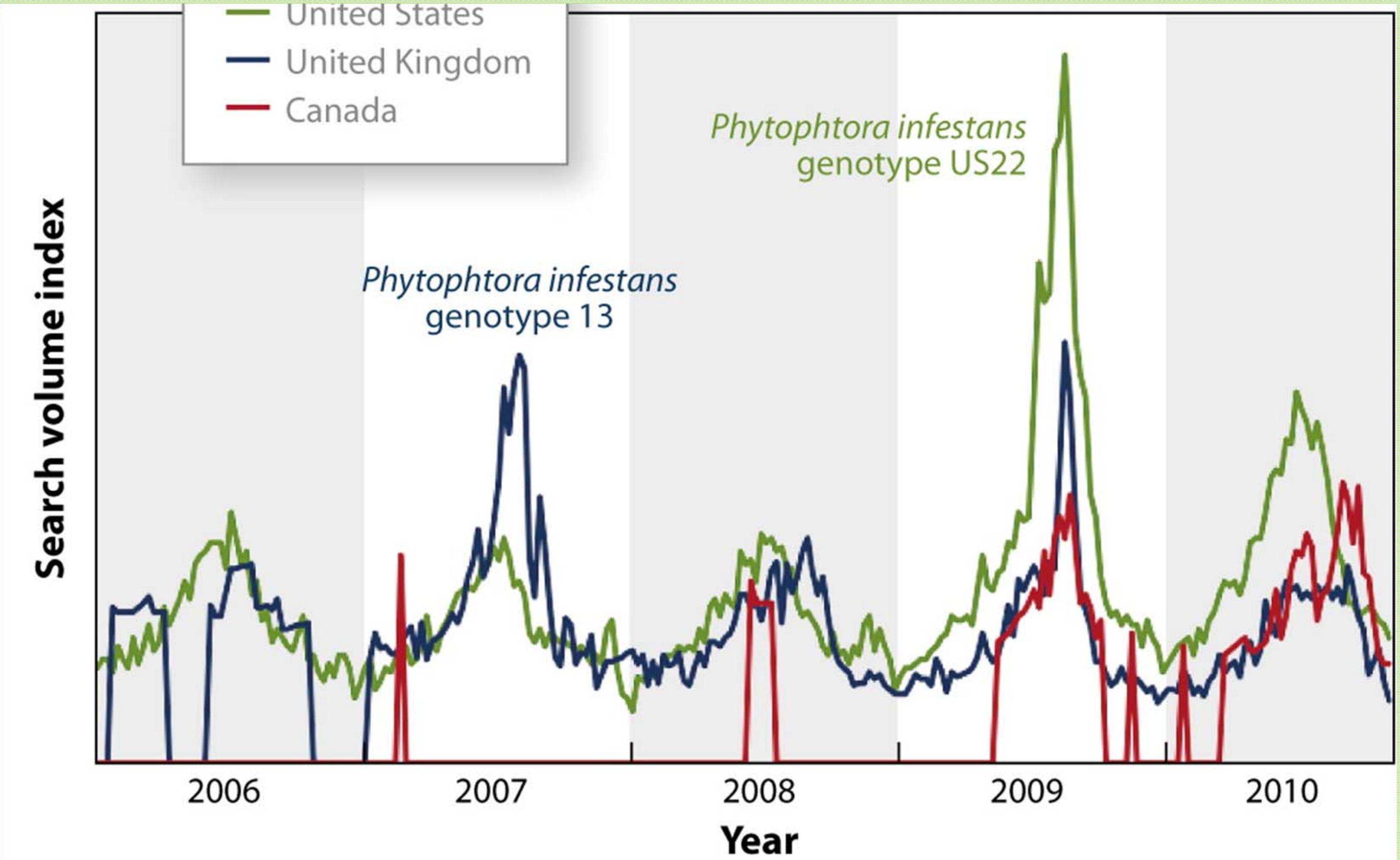
ETI, Example 2: oomycete

ETI – Example 2 - oomycete



Vleeshouwers VGAA, et al. 2011.

Numerous R genes available in potato that confer resistance against late blight

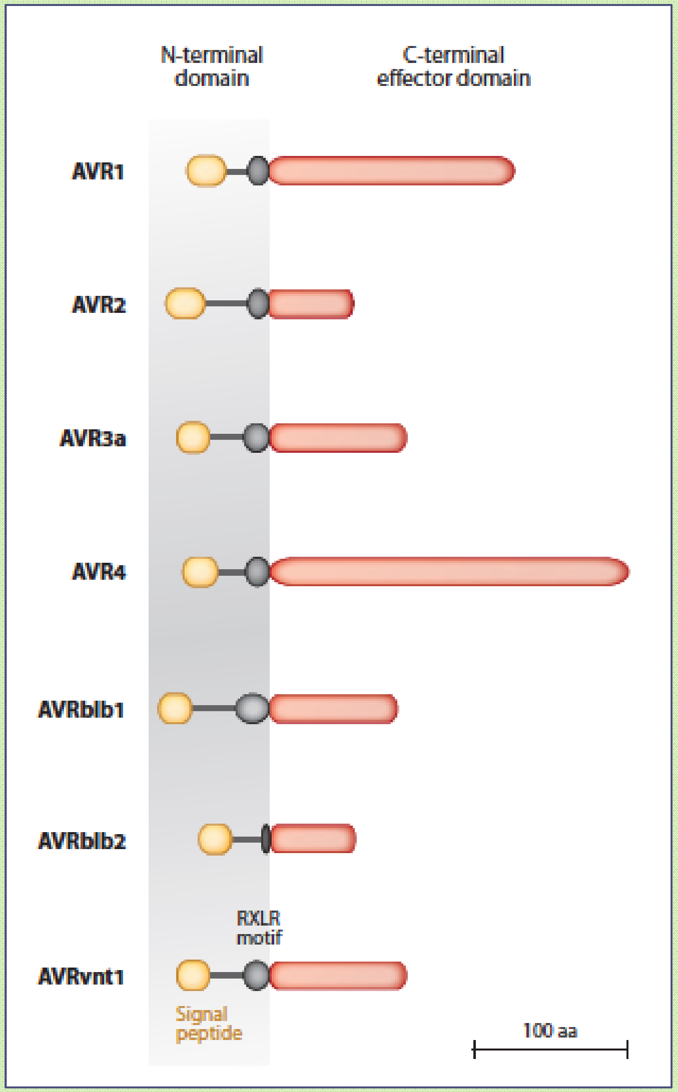
Table 1 *Solanum R* **genes that confer resistance to** *Phytophtora infestans*

R gene	RGH[a]	*Solanum* species[b]	Origin[c]	Chr[d]	Reference
R1 **family**	None				
R1		*demissum*	Mexico	V	(3, 86)
R2 **family**	None				
R2		*demissum*	Mexico	IV	(8, 78, 82)
Rpi-blb3		*bulbocastanum*	Mexico	IV	(78, 95)
Rpi-abpt		Unknown[e]	Mexico	IV	(49, 78, 97)
R2-like		*edinense*	Mexico	IV	(17, 78, 96)
Rpi-edn1.1		*edinense*	Mexico	IV	(17)
Rpi-snk1.1		*schenckii*	Mexico	IV	(17)
Rpi-snk1.2		*schenckii*	Mexico	IV	(17)
Rpi-hjt1.1		*hjertingii*	Mexico	IV	(17)
Rpi-hjt1.2		*hjertingii*	Mexico	IV	(17)
Rpi-hjt1.3		*hjertingii*	Mexico	IV	(17)
Rpi-mcd1		*microdontum*	Argentina	IV	(77)
R3a **family**	*I2* (94)				
R3a		*demissum*	Mexico	XI	(55, 56)
Rpi-sto2		*stoloniferum*	Mexico	XI	(17)
R4 **family**	Unknown[f]				
R4		*demissum*	Mexico	XI	(136, 142)
Rpi-blb1 **family**	None				
Rpi-blb1, RB		*bulbocastanum*	Mexico	VIII	(123, 134)
Rpi-sto1		*stoloniferum*	Mexico	VIII	(145)
Rpi-pta1		*stoloniferum*[g]	Mexico	VIII	(145)
Rpi-blb2 **family**	*Mi* (114)				
Rpi-blb2		*bulbocastanum*	Mexico	VI	(135)
Rpi-vnt1 **family**	*Tm2*[2] (72)				
Rpi-vnt1.1		*venturii*	Argentina	IX	(100)
Rpi-vnt1.2		*venturii*	Argentina	IX	(30)
Rpi-vnt1.3		*venturii*	Argentina	IX	(100)

Vleeshouwers et al. 2011

Features of characterized
Phytophthora Avr gene products

C-terminal domain carries the effector biochemical activity



Vleeshouwers et al. 2011,
Ann Rev Phytopathol 49: 507-

IPM compatible, environmentally sound solutions such as

- PTI
- ETI
- biocontrol
- pheromone-based control measures

are feasible but almost inevitably **knowledge intensive**

Pathogens should be diagnosed early

in most cases at least at species level, and preferably (for ETI) at effector level (‘host race’, ‘pathovar’, ‘pathotype’ etc.)

in a high throughput, affordable and fast manner

DNA-based diagnostics seem most appropriate to reach these goals

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Requirements for building large DNA frameworks:

1. Thorough classical (phytopathological) taxonomical knowledge

(scarce)

2. Molecular expertise

(less scarce)

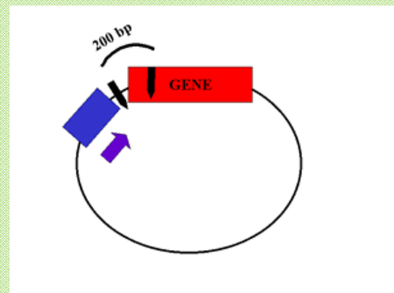
3. Perseverance & some recourses

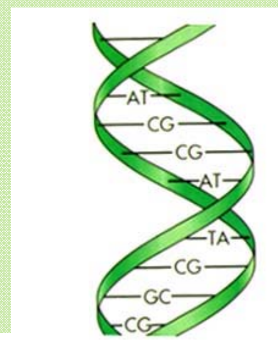
(increasingly scarce)

From morphology to DNA-based diagnostics

Parasitic and non-parasitic nematodes

≈ 2,500 taxa





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Small Subunit Ribosomal DNA-Based Phylogenetic Analysis of Foliar Nematodes (*Aphelenchoides* spp.) and Their Quantitative Detection in Complex DNA Backgrounds

Katarzyna Rybarczyk-Mydłowska, Paul Mooyman, Hanny van Megen, Sven van den Elsen, Mariëtte Vervoort, Peter Veenhuizen, Joop van Doorn, Robert Dees, Gerrit Karssen, Jaap Bakker, and Johannes Helder

Phytopathology 2012

+ assays for cyst, root knot, lesion, stem and stubby root nematodes

Next major step: effector-based pathogen diagnostics

Maximizing the use basal and effector-based plant immunity by match making

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